UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: August 12, 2008

SUBJECT: Response to Deficiencies in Support of the Registration of the Refined Oil of Nepeta Cataria (TGAI)

Decision Number: 371861
DP Number: 351625
EPA File Symbol Number: 71654-EN
Chemical Class: Biochemical
PC Code: 004801
CAS Number: 490-09-5 (dihydronepetalactone)
Active Ingredient Tolerance Exemptions: None
MRID Numbers: 47370401, 47362602, 47362603, 47362604

FROM: Kent R Carlson, PhD, Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Raderrio Wilkins, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

******** CONTAINS CONFIDENTIAL BUSINESS INFORMATION ********

ACTION REQUESTED

E.I. du Pont de Nemours and Company has submitted a response to deficiencies for the TGAI "Refined Oil of Nepeta cataria" (71654-EN) as presented in a letter from Hollis to MEntee (10/16/07). The TGAI is intended for use in the manufacture of a dermally applied insect repellent. In support of the registration of the TGAI, the registrant has submitted product chemistry (MRIDs 47362601 and 47362602), efficacy (MRID 47362603), and toxicology (MRID 47362604) data. These studies have been briefly reviewed in this memo.

RECOMMENDATIONS AND CONCLUSIONS

NOTE TO RAL: Some of the product chemistry certified limits are dependent on the previous information submitted for the EP (ie 2f, 2h, 2i). While a minor consideration, the decision for acceptable TGAI certified limits may be altered if the end-use products are altered substantially (such that the components mentioned become detectable in the EP)

1. Product chemistry and CSF deficiencies for the 7% (71654-EG) and 15% (71654-ER) lotion have not been addressed as requested (Hollis to McEntee, 10/16/07).

2. Product chemistry for the TGAI (MRIDs 47362601 and 47362602) has been addressed.

a. The registrant has submitted CAS Registry numbers for all ingredients on the CSF and placed these after the component descriptor ***as requested by EPA***.

b. The registrant has listed impurities present on a previous CSF (10/12/06; [redacted] on the revised CSF (02/28/08) and has provided documentation of the minor constituents and their hydrogenation products ("other ingredients") expected to be found in the Refined oil of *Nepeta cataria* (MRID 47362602, review below).

EPA previously asked the registrant to present additional minor constituents on the CSF, but realizes that presentation of these numerous impurities and their hydrogenated forms on the CSF would add little regulatory value, since they are not of toxicological significance and are in small/highly variable quantities. For this reason, EPA expects that ***presentation in the submitted MRID (47362602) is ACCEPTABLE*** and will fulfill the intent of identifying critical components/molecules in the Refined oil of *Nepeta cataria* distilled plant extract. The Registrant must add "(see MRID 47362602)" to immediately after the component "Other ingredients" in block 10 of the CSF to account for this.

c. The information on the top row of the CSF where refined oil of *Nepeta cataria* is identified as "active technical grade" has been deleted ***as requested by EPA***.

d. The parentheses around the amounts of the remaining ingredients provided in column 13b of the CSF have been deleted ***as requested by EPA***.

e. The identity of the active ingredients given on the CSF and the product label have been made consistent ***as requested by EPA***.

f. The registrant has based certified limits on ***recommendations from EPA*** that [redacted] would be sufficient for regulatory purposes. The former group brackets concentrations found in both the first and second preliminary analysis performed by Exygen and DuPont. The latter group

has been extracted from the preliminary analysis. EPA considers this range satisfactory for regulatory purposes because the component is undetectable in the final EP products.

g. The lower certified limits for impurities, unreacted starting materials, etc. has been deleted ***as requested by EPA***.

h. The proposed upper certified limit for [redacted] has been altered to reflect the upper part of the range described in preliminary analyses [redacted] ***as requested by EPA***. EPA considers this satisfactory for regulatory purposes because the component is undetectable in the final EP products.

i. The proposed upper certified limit for [redacted] has been altered to reflect the upper part of the range described in preliminary analyses [redacted] ***as requested by EPA***. EPA considers these upper limits to be satisfactory, considering the variation present in source material supply, source material composition, the negligible amount of these components in EP products, [redacted] and the anticipation that no additional toxicity (other than that already associated with dihydronepetalactone) will be associated with these components.

j. Following the addition of other ingredients to the CSF, the registrant has ensured that the % by weight total = 100% ***as requested by EPA***.

k. The registrant has completed blocks 5. and 6. of the CSF ***as requested by EPA***.

3. Physical Properties for the TGAI (MRID 47370401)

a. The registrant has addressed explodability **as requested by EPA**.

b. The registrant must submit storage stability and corrosion characteristics tests upon their completion.

c. The registrant has addressed stability in the presence of different temperatures and metals by discussing the relative impacts that packaging and storage will have on the stability of the product **as requested by EPA**.

d. The registrant has provided a method for the determination of density **as requested by EPA**.

4. Tier I Toxicity studies have previously been termed **ACCEPTABLE** (Gardner to Wilkins 10/04/07; Hollis to McEntee 10/16/07). Even so, DuPont has submitted a discussion (MRID 47362604) that addresses questions EPA had regarding a positive mouse lymphoma assay (review below). This discussion provides **ACCEPTABLE** rationales for the TGAI being non-genotoxic and explores the concept of false test positives and weight of the evidence.

5. Product Performance data have been previously termed **UNACCEPTABLE, but Upgradable** (Fuentes to Wilkins 10/04/07). DuPont has responded to study deficiencies by submitting a supplement (MRID 47362603) to the previous study. ***The supplement has satisfactorily addressed the scientific deficiencies present in the original studies. Ethical issues have still not been resolved however, and may need further review (Classification remains UNACCEPTABLE, but upgradable).*** In particular, ethical questions involve, but are not limited to:

1) The use of employees of Insect Control & Research in mosquito bite-testing,
2) The lack of monitoring information on local mosquito-borne vectors prior to testing,
and 3) other issues identified in a previous review (Fuentes to Wilkins 10/04/07).

NOTE TO RAL: Product performance data is not required for the registration of the TGAI (Refined oil of *Nepeta cataria*). This data is only required for Typical End-use Products associated with pests of public health importance (in this case, mosquitoes and black flies).

Endangered Species Assessment

Two rationales support a **Not Likely to Adversely Affect** (NLAA) decision for the TGAI, Refined Oil of *Nepeta cataria*. These are discussed below.

1) No target or non-target biotic exposure (target or non-target) is expected to result from the manufacture and integration of the TGAI into formulated products.

2) EPA has previously established that Chrysopidae (lacewings) may be adversely impacted by dihydronepetalactone, a molecular component in the Refined Oil of *Nepeta cataria* (Carlson to Wilkins, 10/04/07). To address concerns related to endangered Chrysopidae, EPA has retrieved a list of Chrysopidae (lacewings) from Natureserve (http://www.natureserve.org/explorer/index.htm; *Lomamyia banksi*, *Lomamyia flavicornis*, *Nallachius americanus*, *Nothochrysa californica*, *Oliarces clara*, *Polystoechotes punctatus*, *Sympherobius occidentalis*) and cross-referenced these with threatened and endangered listings posted with the US Fish and Wildlife Service (USFWS; http://ecos.fws.gov/tess_public/ StartTESS.do) and LOCATES (OPP-EFED v. 2.1, March 2006). No species of either lacewings or aphids have been listed as threatened or endangered, so no concerns exist for these particular insects.

STUDY REVIEWS

MRID 47370401 - Supplement to Preliminary Analysis Physical and Chemical Characteristics: In this volume, DuPont re-iterated that deficiencies previously identified with the CSF could be identified point-by-point on the CSF. DuPont further addressed deficiencies associated with "PHYSICAL PROPERTIES". This are identified below and appended to the attached table (Table 1):

a) Explodability - DuPont has identified that the Refined Oil of *Nepeta cataria* has a flashpoint of between 140 and 200°F, is a Class IIIA combustible liquid, and has a very limited potential for explosion hazard.

b) Storage Stability and Corrosion Characteristics - DuPont has stated that these "are addressed in a separate study", but has not identified which study, the completion date or other evidence for review (other than the 14-day results originally submitted). These studies should be submitted upon completion.

c) Stability in the Presence of Metals and Ions - DuPont has reported that the Refined Oil of *Nepeta cataria* is stable in the presence of metals and ions. Even so, it will be stored and packaged in High Density Polyethylene containers to minimize such exposures.

d) Density - DuPont has submitted that density measurements (non-GLP) were performed by using a calibrated Mettler Toledo Densito 30PX instrument with automatic temperature correction.

Table 1. Physical and Chemical Properties for Refined Oil of *Nepeta cataria*			
Guideline Reference No./Property		**Description of Result**	**Methods**
830.6302	Color	Yellow @ 21°C	CCL SOP 10.11
830.6303	Physical State	Liquid @ 21°C	CCL SOP 10.12
830.6304	Odor	Minty @ 21°C	CCL SOP 10.13
830.6313	Stability	Stable @ room and elevated temperatures and in the presence of metals and ions	OPPTS 830.6313
830.6314	Oxidation/Reduction: Chemical Incompatibility	Dihydronepetalactone was relatively stable in solution with metals and metal salts after 14 days at 25°C, with slight decreases at 54°C after 14 days.	Multiple
830.6315	Flammability	>99°C (flashpoint between 140°F and 200°F)	CCL SOP 10.18; ASTM Method No. D56 (Closed Cup)
830.6316	Explodability	Not explosive (Class IIIA combustible liquid)	N/A
830.6317	Storage Stability	At 25 °C dihydronepetalactone content was relatively stable. At 54°C, dihydrocontent decreased approximately 5-10% over a 2 week period. Al, Fe, Al acetate, and Iron (II) acetate had minimal effects.	Gas Chromatography
830.6319	Miscibility	Not applicable, product is not to be diluted in petroleum solvents	N/A
830.6320	Corrosion Characteristics	Guideline study is in progress	Pending
830.6321	Dielectric Breakdown Voltage	Not applicable, product is not for use around electrical equipment	N/A
830.7000	pH	3.97 @ 25°C (1% w/w in deionized water)	CCL SOP 10.17; ASTM Method No. E70
830.7050	UV/Visible Absorption	Not applicable	N/A
830.7100	Viscosity	18.09 mm^2/s (cSt) @ 22°C	ASTM D 445 and D446
830.7200	Melting Range	Not applicable, product is a liquid	N/A
830.7220	Boiling Range	266.0 ± 12.0°C (539.2K)	Mettler FP900 Thermosystem
830.7300	Density/Relative Density/Bulk Density	1.0349 @ 20.6°C	Mettler Toledo Densito 30PX instrument (oscillating body method)
830.7370	Dissociation Constant in Water	Not applicable, required only for pure active ingredient	N/A
830.7550	Partition Coefficient	Not applicable, required only for pure active ingredient	N/A
830.7840	Water Solubility	0.254 ± 0.013 g/L @ "ambient temperature"	OPPTS 7840 Gas chromatograph
830.7950	Vapor Pressure	591, 707, 907. 1100, 1320, and 1630 Pa @ 20, 25, 30, 35, and 40°C, respectively	Terranova 722A diaphragm gauge controller

MRID 47362602 - Supplement: Prediction of minor constituents of Refined oil of *Nepeta cataria* as likely hydrogenation products. In this volume, DuPont summarized *Nepeta cataria* oil composition data from seven oil sources (in five reports). Molecular structures of the compounds and their proposed hydrogenated forms were also provided.

Data from the seven plant accessions were averaged, with "-" and "t" counted as 0.0. These averages are presented below in Table 2:

Table 2. Average % Composition for Minor Components in Extracts of *Nepeta cataria*

Compound (CAS Number)	Average % composition	Compound (CAS Number)	Average % composition
Trans,trans-nepetalactone (490-10-8)	<0.1	Caryophyllene oxide (1139-30-6)	4.87
2,6-dimethyl-2,5-heptadien-4-one (504-20-1)	<0.1	Dihydronepetalactone (490-09-5)	4.53
2-methyl-2,3-dihydroindole (?; 6872-06-6)	<0.1	Cis,cis-nepetalactone (490-10-8)	2.6
Nepetalactam	<0.1	α-humulene (6753-98-6)	1.11
3-hexenyl benzoate (72200-74-9)	<0.1	Nepetalic acid (4581-78-6; 75110-45-1)	0.4
Nonanoic acid (112-05-0	<0.1	E-β-farnesene (502-61-4)	0.4
Dihydroactinidiolide (17092-92-1)	<0.1	Humulene oxide (19888-33-6)	0.39
Iridomyrmecin/isoiridomyrmecin (485-43-8)	<0.1	Piperitone (89-81-6)	0.37
Germacrene D (23986-74-5)	<0.1	3-hexenyl ester (105583-82-2)	0.3
(Z)-β-ocimene (13877-91-3)	<0.1	Myrcene (123-35-3)	0.21
(E)-β-ocimene (13877-91-3)	<0.1	β-elemene (723296-74-0)	0.2
1,8-cineole (470-82-6)	<0.1	Thymol methyl ester (1076-56-8)	0.19
Limonene (138-86-3)	<0.1	Dehydronepetalactone	0.14
Trans,cis-nepetalactone (490-10-8)	42.21	Dimethyl-3,7-oxa-1-bicyclo[3,3,0]oct-2-ene (389599-94-4)	0.14
Cis,trans-nepetalactone (490-10-8)	41.19	Camphor (76-22-2)	0.11
β-caryophylline (87-44-5)	6.43		

Data from the molecular structures section of the discussion (p12-47) were tabulated to compare the extract component and the most likely hydrogenation product. These are presented below in Table 3:

Table 3. *Nepeta cataria* Components and Likely Hydrogenation Products

Compound (CAS Number)	Likely Hydrogenated Compound (CAS Number)	Compound (CAS Number)	Likely Hydrogenated Compound (CAS Number)
Trans,trans-nepetalactone (490-10-8)		Caryophyllene oxide (1139-30-6)	4,9,12,12-tetramethyl-5-oxatricyclo[8.2.0.04,6]dodecane (1209-61-6)
2,6-dimethyl-2,5-heptadien-4-one (504-20-1)	2,6-dimethyl-4-heptanone (108-83-8)	Dihydronepetalactone (490-09-5)	Dihydronepetalactone (490-09-5)
2-methyl-2,3-dihydroindole (?; 6872-06-6)	2-methyl-2,3-dihydroindole (6872-06-6)	Cis,cis-nepetalactone (490-10-8)	
Nepetalactam		α-humulene (6753-98-6)	
3-hexenyl benzoate (72200-74-9)	Benzoic acid hexyl ester (6789-88-4)	Nepetalic acid (4581-78-6; 75110-45-1)	
Nonanoic acid (112-05-0)	Nonanoic acid (112-05-0)	E-β-farnesene (502-61-4; 125037-13-0)	2,6,10-trimethyl-dodecane (3891-98-3)
Dihydroactinidiolide (17092-92-1)		Humulene oxide (19888-33-6)	
Iridomyrmecin/isoiridomyrmecin (485-43-8)	Iridomyrmecin/isoiridomyrmecin (485-43-8)	Piperitone (89-81-6)	(2R)-5-methyl-2-(1-methylethyl)-cyclohexanone (188002-55-3)
Germacrene D (23986-74-5317819-88-8)	1,7-dimethyl-4-(1-methylethyl)-cyclodecane stereoisomer (645-10-3)	3-hexenyl ester (105583-82-2)	
(Z)-β-ocimene (13877-91-3)	2,6-dimethyloctane (2051-30-1)	Myrcene (123-35-3)	2,6-dimethyloctane (2051-30-1)
(E)-β-ocimene (13877-91-3)	2,6-dimethyloctane (2051-30-1)	β-elemene (723296-74-0)	1-ethyl-2,4-diisopropyl-1-methyl-cyclohexane (1756-79-2)
1,8-cineole (470-82-6)	1,8-cineole (470-82-6)	Thymol methyl ether (1076-56-8)	Thymol methyl ether (1076-56-8)
Limonene (138-86-3)	1-methyl-4-(1-methylethyl)-cyclohexane (99-82-1)	Dehydronepetalactone	
Trans,cis-nepetalactone (490-10-8)		Dimethyl-3,7-oxa-1-bicyclo[3,3,0]oct-2-ene (389599-94-4)	[3R-(3α,3aβ,6β,6aβ)]-hexahydro-3,6-dimethyl-1(2H)-pentalenone (120709-97-9)
Cis,trans-nepetalactone (490-10-8)		Camphor (76-22-2)	Camphor (76-22-2)
β-caryophylline (87-44-5)	4,9,12,12-tetramethyl-5-oxatricyclo[8.2.0.04,6]dodecane (1209-61-6)		

MRID 47362603 - Supplement to "Evaluation of the efficacy of personal repellents against mosquitoes in Maine" (MRID 46977424), "Evaluation of the efficacy of personal repellents against mosquitoes in Florida" (MRID 46977425) and "Evaluation of the efficacy of personal repellents against blackflies in Maine" (MRID 47015602). In this volume, DuPont has responded to product performance deficiencies reported previously (Fuentes to Wilkins, May 21, 2007). These deficiencies were listed under **IV PRODUCT PERFORMANCE** as:

a. The registrant has provided detailed discussion on the statistics employed to analyze the data. DuPont used an ANOVA derived from statistical software (GraphPad Prism v.4) to analyze repellency in terms of active ingredient concentration and formulation type. The two-way analyses determined that active ingredient concentration effects were statistically different for both mosquitoes and blackflies and the formulation type effects were different for mosquitoes (Table 4).

Table 4. ANOVA Addressing Active Ingredient Concentration and Formulation Type Effects

Insect	Source of Variation	% of Total Variation	P value
Mosquito	Interaction	6.13	0.0251*
Mosquito	Formulation	21.43	<0.0001***
Mosquito	Active Ingredient Concentration	12.86	0.0015**
Blackfly	Interaction	2.12	0.2455^{ns}
Blackfly	Formulation	2.41	0.2159^{ns}
Blackfly	Active Ingredient Concentration	10.56	0.0112*

*** = extremely significant, ** = very significant, * = significant, ns = not significant

Descriptive statistics (GraphPad Prism v.4) were also used in order to describe field test results (complete protection time in minutes). Mean complete protection times against mosquitoes averaged 4 hours 17 minutes (7% liquid), >6 hours 43 minutes (7% lotion), 6 hours 25 minutes (15% liquid), and >7 hours 7 minutes (15% lotion). Mean complete protection times against blackflies averaged >5 hours 59 minutes (7% liquid), >6 hours 59 minutes (7% lotion), > 7 hours 32 minutes (15% liquid), and >7 hours 34 minutes (15% lotion) (p15). The descriptive statistics are presented in Table 5 and 6.

Table 5. Complete Protection Time for the 7% Formulations as Assessed by Descriptive Statistics

	7% Lotion				7% Liquid			
	FL-mosquito	ME-mosquito	ME-black fly	ME-black fly	FL-mosquito	ME-mosquito	ME-black fly	ME-black fly
N	5	5	5	5	5	5	5	5
Minimum	270	410	338	139	192	27	327	121
25%ile	276	413	344	310	221	151	347	146
Median	282	480	480	480	252	288	476	213
75%ile	469	480	480	480	297	347	480	480
Maximum	480	480	480	480	324	347	480	480
Mean	354	453	425	412	257	257	426	293
StdDev	105	37	75	152	47	132	74	174
Std Error	47	17	33	68	21	59	33	78
Lower 95% CI	224	407	332	222	199	92	335	77
Upper 95% CI	485	499	518	601	316	421	517	509

Normality P value	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10
Passed normality test? (α=0.05)	yes	yes	yes	yes	yes	yes	yes	yes
Skewness	0.30	-0.30	-0.30	-1.1	0.032	-0.91	-0.37	0.22
Kurtosis	-2.2	-2.2	-2.2	-0.92	-1.5	-1.1	-2.1	-2.2

Table 6. Complete Protection Time for the 15% Formulations as Assessed by Descriptive Statistics

	15% Lotion				15% Liquid			
	FL-mosquito	ME-mosquito	ME-black fly	ME-black fly	FL-mosquito	ME-mosquito	ME-black fly	ME-black fly
N	10	10	10	10	10	10	10	10
Minimum	309	480	318	335	281	306	360	177
25%ile	320	480	418	458	295	451	444	448
Median	341	480	476	480	307	480	480	480
75%ile	461	480	480	480	331	480	480	480
Maximum	480	480	480	480	362	480	480	480
Mean	374	480	447	461	314	457	461	443
StdDev	68	0.0	52	46	24	56	38	95
Std Error	22	0.0	17	15	7.5	18	12	30
Lower 95% CI	325	480	409	428	297	417	433	375
Upper 95% CI	422	480	484	494	331	497	488	511
Normality P value	>0.10	-	>0.10	0.03	>0.10	0.0287	0.09	>0.10
Passed normality test? (α=0.05)	yes	-	yes	no	yes	no	yes	yes
Skewness	0.61	0.0	-1.4	-2.0	0.54	-1.9	-1.7	-2.2
Kurtosis	-1.5	0.0	0.77	2.4	-0.78	2.3	1.8	3.2

Landings per count interval were also displayed graphically for each untreated subject (p10, p12, p13). Graphs indicated relatively constant to increasing landing pressure over time.

b. The registrant has addressed the inconsistencies concerning the amount of test material applied to subjects. Reported amounts of applied test materials are as follows in Table 7.

Table 7. Application of Test Material to Subjects

Units	7% Lotion	7% Liquid	15% Lotion	15% Liquid
mg/cm^2	2.56	1.60	2.52	1.61
$g/600cm^2$	1.536	0.960	1.512	0.966
mg/m^2	1792	1116	3780	2412
OPPTS 810.3700 rec. values ($g/600cm^2$)	1.50	1.0	1.50	1.0

c. It is not clear whether the landing rates for the whole body counts are based on 1 minute exposure taken for 1. This information regarding landing rates must be noted in the results table (Appendix IV).

The registrant has provided information regarding the landing rates for the efficacy tests. The treated subjects were exposed to mosquitoes or black flies and watched for 8 hours or until it was judged the repellent had broken down (first of two mosquito bites, or 2 second duration blackfly landings occurring within 30 minutes of each other).

Landing rates on **skin patches** for untreated subjects were taken for 5 minute durations every 30 minutes. The number of mosquitoes or black flies on the untreated person's **body** was also counted for 1 minute durations every hour.

d. The test sites were not monitored for incidences of mosquito-borne disease prior to testing. The registrant has provided additional information regarding the potential for mosquito and blackfly-borne diseases. These factors illustrate the low probability for contracting mosquito or black fly-bourne diseases and that if such occurred, the workers would be covered medically. The rationales for maintaining human safety in this study were as follows:

1) Each subject gave Informed Consent.
2) Each subject was covered in entirety from biting insects, except for the open testing skin patches.
3) Black fly tests were considered completed after landings, not bitings.
4) Mosquito tests were considered completed after 2 mosquito bites within 30 minutes of each other (so the potential total number of bites per day was marginal).
5) Exposure protocols were cleared through DuPont's Human Studies Review Board and an external Institutional Review Board (non-EPA).
6) Each subject's shoes were treated with Permanone® to discourage tick attachment while in the field.
7) There had been no reported human cases of West Nile Virus (WNV) in Maine as of the date of testing.
8) *Culex* spp. mosquitoes (WNV carriers) are uncommon in Maine near the study sites.
9) *Ochlerotatus intrudens* was the only positively identified mosquito in the Maine field trial. This mosquito has not been identified as a WNV carrier by the CDC.
10) *Culex* spp. mosquitoes are uncommon at the Florida site.
11) Potential WNV carrier species including *Psorophora ferox*, *Ochlerotatus atlanticus/tormentor*, *Ochlerotatus taeniorhynchus*, and *Culiseta melanura* were present throughout the FL tests.
12) The potential WNV carrier *Culex iolambdis* was present only late in the study, so probably contributed little to the bite pressure.
13) The subjects were employees of Insect Control & Research and so were covered by Workman's Compensation if medical problems occurred.

MRID 47362604 - Response from DuPont to the U.S. EPA Genetic Toxicity Recommendation in "Science Review and Human Health Risk Assessment in Support of the Registration of the Insect Repellent Refined Oil of *Nepeta cataria* (TGAI), and two lotion end-use products": DuPont has voluntarily submitted a response to EPA concerns regarding a positive mouse lymphoma test (MRID 46977413) and identified potential mitigating circumstances inherent in the study design and proposed a weight of evidence decision for overall effect categorization.

A single positive response was observed in the *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/-Mouse Lymphoma Assay (MLA; OPPTS 870.5300; MRID 46977413). EPA recommended that the registrant confirm this result with an additional assay in a mammalian cell system and/or discuss the significance of the positive finding.

In the rebuttal, DuPont proposed that the four submitted studies were sufficient to address the mutagenic properties of the TGAI, concurred with the assessment that there was "clear evidence of induced mutant colonies over background", but disagreed with the conclusion that this created a genetic toxicology data gap. DuPont further supported this line of reasoning by pointing out that:

1) the positive response in the MLA was observed at doses approaching cytotoxicity (mutagenicity secondary to cytotoxicity),

2) the frequency of small colonies was increased in the MLA test, suggesting that point mutations or deletions were occurring in the TK locus. Point mutations or deletions were not, however, observed in the other *in vitro* tests at comparable doses and conditions (non-reproducible non-confirmable effect),

3) the negative *in vitro* results were confirmed by an *in vivo* mouse micronucleus assay, in which the dose induced adverse systemic effects (decrease in frequency of polychromatic erythrocytes), which suggested that the compound was tested at an appropriate limit dose and was reaching the target site,

4) the high frequency of false positives for the MLA test,
and **5)** the lack of structural or biochemical properties in the TGAI that would be associated with genotoxicity.

When considered in toto, the weight of evidence presented suggests that the TGAI (Refined Oil of *Nepeta cataria*) is non-mutagenic. As such, no further genotoxicity testing is required, and the requirement for higher Tier studies (ie Oncogencity, OPPTS 870.4200) is not triggered.

cc: Roger Gardner, Kent R. Carlson, Raderrio Wilkins, Clara Fuentes, BPPD Chron File, IHAD/ARS

Kent R. Carlson, FT, PY-S: *08/12/08*